

**RAD EXPOSURE REPORT (05/22/19)**

INITIAL REVIEW EXPOSURE REPORT

CBI: No

TERA: R19-0001

ASSESSOR: Lynch

SUBMITTER: Synthetic Genomics, Inc.  
 11149 North Torrey Pines Rd.  
 LaJolla, CA 92037

MICROORGANISMS:

Recipient/host (p. 16):

- i. *Parachlorella* STR00012

Donor (p. 16):

- i. loXP site derived from bacteriophage P1
- ii. Green fluorescent protein "TurboGFP™"

GEM: The TERA is referred to as *Parachlorella* STR26155 (p. 16).

PV (CFU/yr):  $6.0 \times 10^{16}$  CFU/yr

Basis: See Engineering Report for Details

USE: This strain was developed to have virtually no discernable phenotypic differences relative to the recipient (i.e. starting) strain, but which possesses a nucleic acid signature and corresponding reporter protein to allow to the tracking of this specific strain in open-culture and in the environment

SUMMARY:

The submission states that the TERA will be used to collect real-world data on the potential for the algae to disperse, establish, and impact the local environment

The trial will take place at the Synthetic Genomics, Inc. - California Advanced Algae Facility (CAAF) in Calipatria, CA. The TERA will be cultivated in photobioreactors, then outdoor miniponds, and grown to a target density.

When the cell density in the minipond reaches the desired level, the TERA will be inactivated and disposed to an evaporation pond, before ultimately being disposed to a landfill.

#### NOTES AND KEY ASSUMPTIONS

The submitter indicates that once the field experiment has been terminated, all biomass will be inactivated by bleaching the cultures with at least 4 mL/L of 12.5% sodium hypochlorite for at least one hour prior to disposal. All equipment will be cleared of the microorganism (including sample containers, ponds, PBRs, etc.) by bleaching or autoclaving and will be discarded as necessary. Any pond spills will be contained within the secondary containment and treated with bleach. The liquid will then be disposed of into the evaporative pond at the CAAF site (p. 46)

Inactivation studies were performed on STR00010, STR00012, and STR26155. Experimental data showed that 2 mL/L of 4.0% sodium hypochlorite was sufficient to inactivate STR00010 after one hour. STR00012 and STR26155 were inactivated with 1 mL/L of 4.0% sodium hypochlorite after one hour with good mixing. All SGI protocols for inactivation utilize at least 4 mL/L of 12.5% sodium hypochlorite and a minimum contact time of 1 hour to ensure a total deactivated before disposal. Thus, standard SGI CAAF protocols apply greater than a 12.5-fold excess hypochlorite treatment (than that experimentally determined) to inactivate the subject strain providing a conservative treatment for algal cultures (p. 70).

The submitter also indicated that at the end of the toxicant contact time, the vessels were centrifuged to remove any extracellular toxicant, and the pelleted biomass was utilized to inoculate culture into fresh media. These cultures were incubated for one week before examining for growth. An inactivation method was deemed to be effective if after one week of growth, no viable cells were observed in the new culture vessels (p. 69). Therefore, the technical contact stated that they expect 100% inactivation, but indicated a minimum of 7-log inactivation efficiency (see contact report).

RAD assesses a 100% release scenario. After inactivation, the TERA will be sent to an on-site evaporation pond, and subsequently sent to landfill (p. 41).

#### Manufacturing: Laboratory Propagation

Number of Sites/Locations: 1

Synthetic Genomics, Inc.  
11149 North Torrey Pines Rd.  
LaJolla, CA 92037

#### **PROCESS DESCRIPTION:**

The subject microorganism was created within the labs at SGI. The strain is then transported to the SGI La Jolla Greenhouse (within the same research park) in sealed secondary containers. There, the cultures are maintained and scaled prior to movement to the CAAF. Shipment of the subject microorganism will be made in clearly-labelled, sealed containers of approximately one to three liters. These will be further contained in secondary spill-proof containers and transported with enough bleach to neutralize the cultures in the case of a catastrophic failure (p. 44). The technical contact submitted 'SGI Algal biofuels culture scale up process and associated worker exposure' which indicated that the strain will be scaled up to five 40 L carboy then transferred to the Calipatria Research Station (see 'SGI Algal biofuels culture scale up process and associated worker exposure').

#### **ENVIRONMENTAL RELEASE SUMMARY**

Submission did not estimate releases during laboratory or greenhouse propagation. RAD assesses per standard methodology from the Biotech GS.

**WATER:** Negligible

Basis: See Engineering Report for Details

**AIR:** Negligible

Basis: See Engineering Report for Details

LANDFILL: Negligible

Basis: See Engineering Report for Details

INCINERATION: Not expected

Basis: See Engineering Report for Details

PROCESSING/USE: Propagation in PBRs and Open Raceway Ponds

Sites/Locations: 1

Synthetic Genomics, Inc. - California  
Advanced Algae Facility (CAAF)  
250 West Schrimpf Road  
Calipatria, CA, 92233

Days/yr: 98

Basis: See Engineering Report for Details

## **PROCESS DESCRIPTION**

Seed stocks will be maintained in a dedicated grow room and transferred only between sealed containers during the scaling process. Once at least 100 L of seed has grown to a density of at least 1.0 g/L, the seed stock will be utilized to inoculate the 2,000 L and 4,000 L PBRs at a density of approximately 0.1 g/L. Once the PBRs reach a density of at least 1.0 g/L, they will inoculate one of the 0.1-acre ponds at a target operational starting density of 0.1 g/L. These ponds will then run for one week each. At the end of a week of growth, the ponds will be deactivated and disposed (p.40).

PBRs and ponds have secondary containment in the form of a 24-inch berm that is lined with a mesh reinforced, puncture resistant, UV-resistant material. The berm has an effective footprint of 1 acre and can hold the approximately 5x the capacity of the two 0.1-acre L ponds plus all PBRs, in the

highly unlikely scenario of complete primary containment failure (p. 45).

The submitter will regularly sample multiple sample types from a variety of sites (e.g. bioaerosols, trap ponds, CAAF production ponds, local environmental sampling) to provide data on the potential release of the engineered alga from the experimental ponds. The submitter will conduct active monitoring for one week prior to the start of open engineered alga cultivation, during the entire course of the experiment, and for 2 weeks following termination of the engineered alga ponds. During this active monitoring period, one type of endpoint will be the five 350 L "algae-trap" ponds established to help assess the dispersion capability of the subject organism. Additionally, the submitter will sample regularly from all other ponds on site that are in active use and assay for the presence and abundance of the subject strain. Lastly, regular bio-aerosol samples will be collected and similarly assayed for the presence and abundance of the subject strain. Both during the active monitoring, and for one year following first inoculation, the submitter will continue to carry out passive monitoring consisting of monthly sampling from established environmental stations (p.40).

Samples will be collected daily for the CAAF Lab to perform growth measurements. Briefly, these measurements will include optical density (OD730), ash-free dry weight (AFDW), photosynthetic efficiency (PAM), total organic carbon (TOC), fatty acid methyl ester composition (FAME), microscopic analysis and metagenomic analyses. Excess samples will be disposed of in 0.5% sodium hypochlorite. The culture will be inoculated with media containing nitrogen, phosphorus, and trace minerals. (p. 40)

At the end of each experiment, the ponds will be deactivated-in-place with at least 4 mL/L of a 12.5% sodium hypochlorite solution before disposal in the site's evaporation pond. (p. 41)

Clean-in-place procedures are utilized for cleaning ponds at the CAAF site. At the conclusion of an experiment, ponds are scrubbed along the sides with brushes to remove any films that may have formed over the course of an experiment. Then, ponds are dosed with 4 mL/L of 12.5% sodium hypochlorite and thoroughly mixed with the in-pond paddlewheels. After at least one hour, and after complete mixing, the ponds are then pumped directly to the on-site evaporative disposal pond via a dedicated line. (p. 70).

## ENVIRONMENTAL RELEASE SUMMARY

To ensure that the subject microorganism is completely removed from the test site after the experiment has been completed, all liquid biomass will be treated with 4 mL/L of 12.5% sodium hypochlorite for at least one hour prior to disposal. This dose is 12.5-fold greater than the experimentally determined effective dose for killing both recipient and subject strains. Scale up vessels, including Fernbach flasks and carboys, will be treated with bleach to neutralize the microorganism before dumping down the drain to the evaporative pond. Carboys will be cleaned and autoclaved for reuse. 0.1-acre ponds will be deactivated in place with bleach before disposal into the evaporative pond. Samples that have been collected from the site will be neutralized by treatment with 4 mL/L of 12.5% sodium hypochlorite for a minimum of one hour before disposal (p. 45).

### WATER:

Amount: negligible

Basis: See Engineering Report for Details

### LANDFILL (from evaporation pond):

Per submission, all process liquid waste is piped to an evaporation pond with a total capacity of 8.6 acrefeet (AF). The pond is permitted by the California Water Quality Control Board Region #7. The pond was designed to comply with Federal, State and County construction standards. Quarterly Reports on the evaporation pond physical integrity, chemical composition and water levels are provided to the State. (p. 42)

Evaporated salt waste material that is >50% water can be shipped via licensed hauler in lined dump trucks to a licensed Class-II landfill for disposal (lined to contain liquids). However, the preferred means of disposal will be to allow the material to dry below 50% water, and when the dried material passes the EPA "paint filter test" it will be shipped via a licensed vender in unlined trucks to a licensed Class-III landfill. A Special Waste Profile has been approved by a local landfill. (p.42)

1) From: PBR cleaning

Amount: negligible

Basis: See Engineering Report for  
Details

2) From: Pond/Equipment Cleaning

Amount:

$1.2 \times 10^8$  CFU/yr  
 $1.0 \times 10^7$  CFU/day over 12 days/yr

Basis: See Engineering Report for  
Details

3) From: Unused PBR Biomass Termination

Amount: negligible

Basis: See Engineering Report for Details

4) From: Pond Termination

Amount:

$5.9 \times 10^9$  CFU/yr CFU/yr  
 $4.9 \times 10^8$  CFU/day over 12 days/yr

Basis: See Engineering Report for Details

AIR:

1) From: Bioaerosol emissions

Amount:

$6.0 \times 10^7$  CFU/yr  
 $7.1 \times 10^5$  CFU/day, over 84 days/yr

Basis: See Engineering Report for Details

2) From: Fugitive Emissions During Sampling

Amount: Negligible

Basis: See Engineering Report for Details

INCINERATION: Not expected

Basis:

See Engineering Report for Details

## **CONSUMER and GENERAL POPULATION EXPOSURE**

### **Consumers**

The TERA is not intended for use in consumer products. Therefore, exposure to consumers is not expected.

### **General Population**

There are releases of the TERA to air and landfill from processing/use. However, landfill disposal regulations (state and federal), landfill design and management practices and low emissions to air are expected to mitigate the exposures to negligible levels.

### **Exposure to Releases from Processing/Use.**

#### **Inhalation Exposure**

#### **Bioaerosol Fugitive Emissions**

To estimate exposures from this source the Gaussian algorithm described in Turner (1970) was used. The scenario assumes a release height of 10 meter; a 100 meter receptor distance from the source , a wind speed of 5.5 m/sec and a neutral atmospheric stability.

The equation for a continuous release is as follows:

$$\text{Conc} = Q \quad \text{yr} \times 3.17 \times 10^{-12}$$

Since it is unlikely that, as a reasonable worst case, the wind blows in one direction. It is more reasonable to assume that the wind blows in one direction 25 percent of the time; therefore, the corrected equation is.

$$\text{Conc} = Q \text{ yr} \times 7.9 \times 10^{-13}$$

where:

Conc = Concentration in ambient air (CFU/m<sup>3</sup>)

Q yr = Release rate (CFU/yr)

**Using the estimated maximum release of  $6.0 \times 10^7$  CFU/yr the concentration in ambient air 100 meters downwind would be much less than 1 CFU/m<sup>3</sup>.**

The exposure from this concentration would be calculated as follows:

$$\text{Exposure} = C \times \text{IR} \times \text{ET} \times \text{AT}$$

where:

C = concentration in ambient air (CFU/m<sup>3</sup>)

IR = Assumed inhalation rate of 1.1 m<sup>3</sup>/hr

ET = Exposure time is 24 hr/day

AT = Averaging time is 365 day yr

**Thus exposures are expected to be negligible (<1 cfu/yr ).**

**It should be noted that examination of satellite imagery of the Processing/use site indicated the closest residences to be approximately 1.5 miles from the site.**

#### **REFERENCES:**

Hollinshead, W. 2019. Initial Review Engineering Report for R-19-0001

Synthetic Genomics, Inc. 2019. TSCA Experimental Release Application R-19-0001